Histological Differentiation Among Abiotic Causes of Conifer Needle Necrosis

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ACKNOWLEDGMENTS
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RESEARCH SUMMARY
Histological analyses of pollutant-caused necrosis of Pinus ponderosa and Pseudotsuga menziesii current-year foliage lab-inoculated with hydrogen fluoride, sulfur dioxide, and ethyl mercaptan, showed that necrosis caused by phytotoxic gases can be differentiated from that induced by winter drying, drought, and salt. Hypercyst and hyperplasia of vascular parenchyma endodermis collapse, and intense vascular staining are characteristic of injury caused by the pollutants in both species, but were not found in needles injured by the other causes.

Similar analyses were done on necrotic Pinus contorta and Pseudotsuga menziesii conifer foliage collected in the field near industrial sources of fluoride, sulfur dioxide, mercaptans, and hydrogen sulfide and from specimens collected from sites known to have been injured by winter drying. Species included Pinus contorta, Pinus ponderosa, Pseudotsuga menziesii, Pinus sylvestris, and Picea pungens. As in the laboratory fumigations, winter drying injury was readily distinguished from that caused by phytotoxic gases. The internal symptoms caused by industrial fumigations were similar to those induced by gases under controlled conditions and symptoms of winter drying from field-collected specimens were similar to those simulated in the laboratory. These results differ substantially from conclusions reached in a similar study in 1973. The present study shows that histological analysis should be useful in diagnosing air pollution-induced injury and damage in coniferous forests.

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INTRODUCTION

Conifer needle necrosis and chlorosis can be induced by several agents including salt (Spotts and others 1972); winter drought (Carlson and Meyer 1973); air pollutants including fluoride, sulfur oxides, reduced sulfurs (Treshow and Pack 1970; Carlson and others 1974; Weinstein 1977); and presumably other causes. Field studies of air pollution-related injury and damage to conifers near emitting sources involve a great deal of subjectivity in the identification of cause. Even though foliar chemical analyses may indicate accumulation of a pollutant in a forest, relationships of needle abnormalities to the contaminant may remain obscure (Treshow 1969). Solberg and Adams (1956), Carlson and Dewey (1971), and Gordon (1972) suggested that light microscopy of sections of affected needles may be diagnostic. Sections were made in a transition zone defined as a ca. 0.08 in (2 mm) needle segment that includes healthy, chlo­roptic, and necrotic tissue. However, Stewart and others (1973) claimed that fluoride, salt, and winter damage caused virtually identical histological changes within symptomatic needles.

The purpose of this research was to determine whether abiotic causes of necrosis induce differential symptomatology in conifer needle tissue; the work involved laboratory and field studies.

METHODS

Laboratory Study

Two-year-old Pseudotsuga menziesii and Pinus ponderosa seedlings were obtained in late winter from the Forest Service nursery at Coeur d'Alene, Idaho, transplanted while dormant to 8-inch clay pots, and placed in a greenhouse. The potting medium was 70 percent nursery soil and 30 percent composted sawdust. No fertilizer was added, and pots were watered evenly every 3 or 4 days. Greenhouse temperature and humidity were recorded on a hygrothermograph throughout the experiment. The first 2 weeks following transplanting, greenhouse temperature was kept at ca. 50° F (10° C) and photoperiod at 16 hours with 550 fc (foot-candles) to minimize transplant shock. Temperature then was increased to 70° F (21° C) during the day and was allowed to drop to 50° F (10° C) at night. Bud break occurred ca. 3 weeks after transplanting. Needle development appeared normal and there was no visible evidence of transplant shock. Four months after transplanting, new foliage was fully elongated and the seedlings were physiologically active. By this time, temperature fluctuated diurnally between 50° to 90° F (10° to 33° C) and light intensity varied between 500 and 6,000 fc, depending on cloud cover. Relative humidity varied from 45 to 85 percent, averaging 65 percent. Seedlings did not exhibit adverse effects due to greenhouse environmental conditions.

Several phytotoxic gases commonly emitted from industrial sources located near coniferous forests and other abiotic stresses often suggested as alternative causes of foliar injury were selected as treatments. Seedlings of each species were randomly segregated into eight groups of ten each. Each group of ten seedlings was subjected to one of the following randomly assigned treatments: control, excessive salt, drought, simulated winter drying, sulfur dioxide (SO2), hydrogen sulfide (H2S), ethyl mercaptan (C2H6S), and hydrogen fluoride (HF). All treatments except controls were administered specifically to develop foliar injury; when injury was noted seedlings were removed to the normal greenhouse environment. Treatments are detailed below.

CONTROL

Seedlings were placed in a stainless steel chamber of 18 ft3 (.51 m3) internal volume with plexiglass roof and windows. The chamber was refrigerated to maintain relatively constant temperatures and light was provided with a bank of fluorescent and incandescent lights emitting about 1,500 fc at tree level. Temperature was maintained at 68° to 77° F (20° to 25° C) and relative humidity at 50 to 60 percent.

Charcoal-filtered air was supplied to the chamber through a Worthington air compressor at ca. 1.77 ft3/min (50 l/min) for 72 hours. Seedlings were then removed to the normal greenhouse environment.
SALT
Seeds were placed in a separate watering tray and watered every 2 to 3 days for 7 weeks with 1.2 percent (12,000 ppm) solution of NaCl according to Spots and others (1972). Salt-free water then was applied for the duration of the experiment.

DROUGHT
Water was withheld from the seedlings for 22 days, at which time needle showed obvious visible symptoms of moisture stress. Normal watering then was resumed.

SIMULATED WINTER DRYING
A short of 1/2 -inch (1.2 -cm) -thick plywood was fitted to the top of a chest-type freezer. Holes were cut large enough in the wood to allow insertion of the lower portion of the pots; the pot lip prevented the container from falling through the hole. The freezer lid was left open, the board was placed over the opening, and the pots with seedlings were inserted (fig. 1). Freezer temperature was maintained at 0 °F (-18 °C), effectively freezing the soil and seedling roots while the tops remained at greenhouse temperature. Soil and freezer temperatures were monitored daily. After 3 days of freezing, a small oscillating fan was placed about 8.3 ft (2.5 m) from the freezer; the fan cost a light breeze over the seedlings. Except for the relatively high greenhouse temperature, this treatment simulated winter drought conditions. Foliar chlorosis appeared 2 days later and the treatment was discontinued. The pots then were placed in the normal greenhouse environment.

HYDROGEN SULFIDE
Hydrogen sulfide was obtained from Matheson in a pressurized tank at 1.20 percent in helium. The gas was delivered to the seedlings at 50 ppm for 8 hours as described for SO₂.

ETHYL MERCAPTAN
Ethyl mercaptan was obtained from Matheson at 1.28 percent in pure nitrogen and administered to the plants for 10 hours at 50 ppm as described for SO₂.

HYDROGEN FLUORIDE
Hydrogen fluoride at 113 ppm in air was obtained from Matheson and delivered to the plants at 5 ppm as described for SO₂. Injury appeared within 3 hours and the seedlings were removed from the chamber to the normal greenhouse environment.

Table 1.—Apparatus used to induce winter drought temperatures, freezing the soil and root systems, while the seedlings were maintained at greenhouse temperature. A light breeze generated by a small fan directed over the seedlings completed the winter drying simulation.

SULFUR DIOXIDE
Seedlings were placed in the chamber described for the control treatment. Sulfur dioxide was obtained from Matheson Gas Co. in a pressurized tank at 1 percent in air. Tank SO₂ was diluted with charcoal-filtered air through a mass flowmeter to achieve 5 ppm 1/3 in the airstream to the chamber. Air was supplied through a Worthington air compressor; flow was measured with a Matheson 6005 Flowmeter. Chamber concentrations were not directly measured for sulfur dioxide, but for any of the other gas treatments. Corrections for barometric pressure and air temperatures were made as 1/3. Flow rate was maintained at 1.77 ± 0.03 cfm (30 L/min) until symptoms appeared on the needles, about 6 hours later. Seedlings then were removed to the normal greenhouse environment.

Field Study
To determine whether conifer foliage in field situations developed symptoms similar to those observed in the laboratory study, representative necrotic needles of various ages were collected from trees near seven industries known to emit phytotoxic gases and from trees in two areas damaged by winter drying. Sampling locations, major biotic agents, and species are shown in table 1.

Necrotic needles in fluoride-polluted ecosystems were collected near an aluminum plant at Columbia Falls, Mont.; near two aluminum plants in the Rhone Valley of Switzerland; and near a phosphorus plant at Ramsay, Mont. Conifer needle samples within sulfur dioxide-polluted areas were collected near a lead smelter at Helena, Mont., and a copper smelter at Anaconda, Mont. Needles presumably injured by hydrogen sulfide were collected near a geothermal complex in California. Conifer foliage injured by a complex of sulfur dioxide, hydrogen sulfide, and methyl mercaptan was collected near a pulp and paper mill at Missoula, Mont. Specimens representing winter injury were collected in the Blackfoot Valley ca. 20 miles east of Missoula, Mont., and near East Glacier, Mont.

Presence of airborne phytotoxic gases near the industrial sources was assumed. The assumption was based on the publications cited below and on personal knowledge. Air samples were not taken for pollutant analysis at the time foliage samples were collected. Fluoride emissions from an aluminum plant in northwestern Montana were described by the State of Montana (1974). Wood (1968) discussed fluorides emitted by phosphorus manufacturing facilities and the role of sulfur dioxide from copper and lead smelting operations. Hydrogen sulfide released from geothermal energy production in California was described by Miller (1968) whereas reduced sulfurs from a pulp and paper mill in western Montana were described by Berg and others (1973). Calcium and Meyer (1973) documented the occurrence of the winter injury episode in the Blackfoot Valley in western Montana.

Field-collected specimens were 2 to 3 years old because little necrosis was found on first-year needles, whereas current-year foliage was sampled in the laboratory study. The number of field-collected specimens sectioned and observed by causal agent were: fluoride, 64; sulfur dioxide, 16; reduced sulfurs, 30; winter damage, 4; and control, 20. All collected specimens were embedded, sectioned, and stained as described for the laboratory study.
RESULTS AND DISCUSSION

Laboratory Study
Pinus ponderosa and Pseudotsuga menziesii responded nearly identically within treatments and the symptoms (responses to treatment) described below apply to both species. Unless otherwise stated, all observations in the laboratory study refer to the chlorotic region within the transition zone on current-year foliage. Figures 2 through 5 show macroscopic responses of both species to control and sulfur dioxide fumigations.

CONTROL

Needles remained green and healthy throughout the experiment (figs. 2, 4). Cells of all internal tissues appeared turgid and normal, with little or no plasmolysis caused by the histological procedures (fig. 6). Plastids were dispersed within the chloroplasts and were not granulated (table 2 and fig. 7). Xylem cells were stained light green and living cells light green-blue. Staining was normally differentiated with no obvious accumulation or intensification within the vascular tissues (fig. 8).

SALT

Needles showed symptoms of salt toxicity within 15 to 20 days following the initial treatment. Chlorosis appeared at the tip, middle, or base and was independent of species or individual. Usually the transition zone was diffuse, not distinct. In all cases, symptoms intensified until the trees were dead, even though fresh water replaced the salt treatments soon after the onset of symptoms. Mesophyll chloroplasts plasmolyzed extensively and most plastids were destroyed (figs. 9, 10). Endodermal cells in the region of plasmolysed mesophyll remained turgid and intact but xylem, phloem, and transfusion parenchyma collapsed (table 2). Phloem elements virtually disintegrated, and there was no obvious accumulation of stain within the transition zone (fig. 10).

DROUGHT

Chlorosis more often appeared first at the needle base and progressed acropetally. No distinct transition zone was formed. Mesophyll cells collapsed but did not plasmolyze as in the salt treatment (table 2). All other living cell types collapsed except the endodermis (fig. 11). No obvious accumulation of stain occurred within the transition zone (fig. 12).

WINTER DRYING

The syndrome for winter drying (table 2) was virtually the same as for drought except that injury appeared much sooner, within 3 to 5 days (figs. 12, 13).

SULFUR DIOXIDE

Chlorosis appeared near or at the tips within 3 to 8 hours following fumigation and an abrupt, distinct transition zone developed (figs. 3, 5). Sporadic collapse of the mesophyll occurred, and plastids clumped near the plasmolysed area. Endodermal cells in contact with a necrotic mesophyll chloroplasts collapsed (figs. 14, 15). Xylem, phloem, and transfusion parenchyma and phloem elements showed extensive hypertrophy and hyperplasia. Epidermal cells hyperplotted, occluding the resin canals (fig. 16). An intense, purple-red stain developed in the vascular tissues and extended basipetally into the region of non-damaged mesophyll (table 2).

HYDROGEN FLUORIDE, HYDROGEN SULFIDE, AND ETHYL MERCAPTAN

The internal syndrome induced by these pollutants was indistinguishable from that caused by SO2 (table 2 and figs. 17-19). Hydrogen sulfide caused necrosis to only the needle tips, whereas F- and CH3SH injury initially appeared slightly below the tips and progressed acropetally and basipetally. Also, tracheal cells were stained yellowish in needles injured by H2S, unlike the light red in needles injured by the other gases.

Field Study

Control specimens were histologically similar to those of the laboratory study (fig. 20). Similarly, symptoms in the transition zone of field-collected needles within polluted areas were identical with symptoms induced by gaseous pollutants in the greenhouse study (figs. 23-28). Plastids clumped in the mesophyll parenchyma; endodermis collapsed when in contact with damaged mesophyll; and phloem, xylem, and transfusion parenchyma and epithelial cells divided excessively and became abnormally large. Resin ducts were occluded by the hypertrophy of epithelial cells. An intense purple-red stain developed in the vascular tissue extending well into the area of nondamaged mesophyll. The total syndrome was similar regardless of pollutant or species; for example, it was not possible to distinguish fluoride injury from SO2 and all species and needle age responded similarly.

Winter drying, however, was dissimilar to pollutant injury; this difference also was observed in the laboratory study. Mesophyll collapsed, endodermis remained turgid even when in contact with collapsed, necrotic mesophyll, and no hypertrophy or hyperplasia occurred in the parenchyma (figs. 21, 22). Epithelial cells in winter-dried specimens were hypertrophied, often occluding the resin canals. No intense stain developed in the vascular cylinder such as occurred with gas-injured specimens; the symptoms were similar among the different species and needles of various ages.

Histological differentiation of needle necrosis has not been clearly defined. Solberg and others (1955) and Solberg and Adams (1956) showed that HF and SO2 disrupted vascular tissues and caused hypertrophy and hyperplasia of vascular parenchyma. They indicated that HF injury could be distinguished from SO2 by Evans and Miller (1957) stated that SO2, suspected winter injury, and ozone injury could be distinguished histologically in that SO2 disrupted and dissolved cytoplasmic constituents of all needle tissues, whereas ozone injured only the plicate parenchyma and winter flake caused abnormalities within the phloem and transduction cells. Their sections were taken adjacent to necrotic lesions and not within a transition zone as described in this paper. Also, their winter injury was suspected to be direct cold injury and not of the drying type. Stewart and others (1973) were not able to distinguish between symptoms of SO2, HF, and winter injury. They observed hypertrophy of pitted cells and mesophyll parenchyma cells when natural senescence, drought, and fluoride were causes of necrosis. Consequently, Stewart and others (1973) saw little value in the use of histological inter pretations to differentiate among various environmental stresses. Conversely, biotic causes usually are easily diagnosed histologically by signs of the causal organism.

Table 2. Qualitative comparison of experimentally induced abiotic stresses on various Pseudotsuga menziesii and Pinus ponderosa needle tissu es

<table>
<thead>
<tr>
<th>Treatment</th>
<th>External symptomology</th>
<th>Mesophyll</th>
<th>Endodermis</th>
<th>Transfusion parenchyma</th>
<th>Xylem parenchyma</th>
<th>Phloem</th>
<th>Phloem parenchyma</th>
<th>Epidermal cells</th>
<th>Staining pattern in tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Needles or branches</td>
<td>Fully turgid, plastids not phloem, dispersed</td>
<td>Intact</td>
<td>Turgid</td>
<td>Turgid</td>
<td>Turgid</td>
<td>Turgid</td>
<td>Turgid</td>
<td>No deep coloring, xylem xanthophyll, xylem green, chloroplasts destroyed</td>
</tr>
<tr>
<td>SO2</td>
<td>Chlorosis of base, middle, or tip within 15-20 cm. damage zones between necrotic and green tissue not distinct</td>
<td>Extensive pleomorphism, chlorenchyma destroyed</td>
<td>Intact, turgid</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Dris- and end</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Normal, no peripheral concretions of xylem</td>
</tr>
<tr>
<td>HF</td>
<td>Chlorosis of base, middle, or tip within 15-20 cm. damage zones between necrotic and green tissue not distinct</td>
<td>Chlorosis of base, middle, or tip within 15-20 cm. damage zones between necrotic and green tissue not distinct</td>
<td>Intact, turgid</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Dris- and end</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Normal, no peripheral concretions of xylem</td>
</tr>
<tr>
<td>Ozone</td>
<td>Chlorosis of base, middle, or tip within 15-20 cm. damage zones between necrotic and green tissue not distinct</td>
<td>Chlorosis of base, middle, or tip within 15-20 cm. damage zones between necrotic and green tissue not distinct</td>
<td>Intact, turgid</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Dris- and end</td>
<td>Collapsed</td>
<td>Collapsed</td>
<td>Normal, no peripheral concretions of xylem</td>
</tr>
</tbody>
</table>

Note: Observations were made within the transition zone, a 2-mm segment including the necrotic, chlorotic, and green portions of injured needle. Generally, the transition zone is chlorotic. 

1. Feudner’s and Feudner’s green schedule.
Figure 2.—Lab study. Control ponderosa pine seedling after treatment with clean air in fumigation chamber. Current-year foliage remained green; no necrosis or chlorosis was observed.

Figure 3.—Lab study. Ponderosa pine seedling after treatment with 5 ppm SO$_2$ for 6 hours. All current foliage developed necrosis.

Figure 4.—Lab study. Control Douglas-fir seedling. Similar to ponderosa pine, current-year foliage remained green and appeared healthy.

Figure 5.—Lab study. Douglas-fir foliage after fumigation with 5 ppm SO$_2$ for 6 hours. Note the well-defined tip necrosis. Needle segments for histological study were taken from the transition zone, which included green, chlorotic, and necrotic tissue. Serial longitudinal and transverse thin sections $4.72 \times 10^{-4}$ in (12 microns) thick then were made through the entire transition zone. Histological interpretations were made from these sections.
Figure 6.—Lab study. Control, ponderosa pine, longitudinal section, X300. Endodermis (EN), vascular parenchyma (VP), phloem (P), and xylem (X) are fully turgid. Hypertrophy and hyperplasia of parenchymatous tissue have not occurred here, but do in needles affected by phytotoxic gas. Current-year foliage.

Figure 7.—Lab study. Control, ponderosa pine, transverse section, X300. Note position of epidermis (ED), hypodermis (HY), mesophyll parenchyma (MP), and resin canal (RC). Presence of numerous dispersed plastids in turgid mesophyll cells and nonhypertrophied epithelial cells (EP) in the resin canal are indicative of a healthy needle. Current-year foliage.

Figure 8.—Lab study. Control, ponderosa pine, transverse section, X300. Note position of phloem (P), vascular parenchyma (VP), xylem (X), endodermis (EN), and mesophyll (MP). Endodermis is turgid and phloem and vascular parenchyma are not deeply stained nor hypertrophied. Mesophyll is turgid and packed with plastids. Current-year foliage.

Figure 9.—Lab study. Salt injury, ponderosa pine, longitudinal section, X300. Mesophyll parenchyma (MP) collapsed, but the endodermis (EN) remained turgid even when in contact with necrotic mesophyll cells. Note the junction between necrotic mesophyll and healthy endodermis (arrow). Current-year foliage.

Figure 10.—Lab study. Salt injury, ponderosa pine, transverse section, X300. This section is from the necrotic portion of the transition zone. Parenchymatous tissues including mesophyll (MP), vascular parenchyma (VP), and phloem (P) collapsed. Even in this condition of extreme injury, the endodermis (EN) is, for the most part, somewhat turgid. Current-year foliage.

Figure 11.—Lab study. Drought, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed but endodermis (EN) in contact with necrotic mesophyll remains turgid. Current-year foliage.
Figure 12.—Lab study. Winter drying, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed, but endodermis (EN) remained turgid. Note similarity between injury by salt, drought, and winter drying (figs. 9–12). Current-year foliage.

Figure 13.—Lab study. Winter drying, ponderosa pine, transverse section, X300. Mesophyll (MP), vascular parenchyma (VP), and phloem (P) collapsed while endodermis remains turgid. No deep staining occurred in the vascular tissues. Current-year foliage.

Figure 14.—Lab study. Sulfur dioxide, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed and endodermis (EN) in contact with collapsed necrotic mesophyll also collapsed. Note the intense reddish staining in the vascular bundle within the endodermis extending basipetally into the region of noninjured mesophyll. Note the hypertrophy (excessive cell enlargement [HT] and hyperplasia (excessive cell division [HP]) that occurred in the vascular parenchyma, causing the vascular bundle to swell. These three symptoms, endodermis collapse, intense vascular stain, and swelling, are characteristic of injury induced by phytotoxic gas, but not by other causes. Compare with salt, drought, and winter drying (figs. 9–13). Current-year foliage.

Figure 15.—Lab study. Sulfur dioxide, ponderosa pine, transverse section, X300. This section is from the green end of the transition zone. Vascular parenchyma (VP) hypertrophied and the characteristic deep reddish stain pervaded the vascular tissues. Mesophyll (MP) has not collapsed. Phloem (P) has been destroyed. Current-year foliage.

Figure 16.—Lab study. Sulfur dioxide, ponderosa pine, transverse section, X300. Epidermal cells (EP) hypertrophied and occluded the resin canal (RC). Mesophyll (MP) is not noticeably affected. Hypertrophy of epidermal tissue commonly occurs in needles injured by phytotoxic gases, but also is found in needles injured by winter drying and drought. It is not distinctive for phytotoxic gases. Current-year foliage.

Figure 17.—Lab study. Hydrogen sulfide, ponderosa pine, longitudinal section, X300. Collapsed endodermis (EN), deep-red vascular staining, and hypertrophy of vascular parenchyma (VP) are evident. These symptoms are similar to those caused by SO₂. Current-year foliage.
Figure 18.—Lab study. Ethyl mercaptan, ponderosa pine, longitudinal section, X300. Symptoms are similar to those caused by sulfur dioxide. Note hypertrophied vascular parenchyma (VP), intense reddish staining in vascular bundle, and endodermis (EN) collapse in contact with necrotic mesophyll (MP). Current-year foliage.

Figure 19.—Lab study. Hydrogen fluoride, Douglas-fir, longitudinal section, X300. Note collapse of endodermal cell (EN) in contact with necrotic mesophyll (MP). Also, the vascular bundle is deeply stained. Symptoms are similar to those caused by sulfur dioxide, hydrogen sulfide, and ethyl mercaptan in ponderosa pine needles. Compare with figures 14–18. In this experiment, the different symptoms induced by various treatments in ponderosa pine needles were identical to those induced in Douglas-fir needles. Current-year foliage.

Figure 20.—Field study. Control, ponderosa pine, transverse section, X125. Note position of mesophyll (MP), endodermis (EN), phloem (P), xylem (X), and vascular parenchyma (VP). The only difference between this needle and the control needle of the lab study (fig. 8) is that the phloem and xylem are better developed. All needles shown for field study were 2 years old.

Figure 21.—Field study. Winter drying, ponderosa pine, longitudinal section, X25. Note collapsed mesophyll (MP). Endodermis (EN) in contact with collapsed mesophyll is turgid, healthy, and vascular parenchyma (VP) is normal. This section included some epithelial (EP) tissue that also appears normal.

Figure 22.—Field study. Winter drying, ponderosa pine, longitudinal section, X25. Note collapsed mesophyll (MP). Endodermis (EN) in contact with collapsed mesophyll is turgid, healthy, and vascular parenchyma (VP) is normal. This section included some epithelial (EP) tissue that also appears normal.

Figure 23.—Field study. Sulfur dioxide, ponderosa pine, longitudinal section, X25. Deeply stained vascular tissue (VT), collapsed mesophyll (MP), and necrotic endodermal cells (EN) occurred. This is similar to specimens injured by sulfur dioxide in the lab study.
Figure 24.—Field study. Sulfur dioxide, ponderosa pine, transverse section, X125. Deep reddish stain (arrow) and necrotic endodermis (EN) in contact with necrotic mesophyll (MP) typify injury by phytotoxic gases.

Figure 25.—Field study. Hydrogen sulfide, ponderosa pine, longitudinal section, X125. Note necrotic endodermis (EN), intense stain in vascular tissue (VT), and hypertrophy of vascular parenchyma (VP).

Figure 26.—Field study. Hydrogen sulfide, ponderosa pine, transverse section, X125. This section is from the greenish portion of the transition zone. Note destruction and heavy staining in the vascular tissues (VT). Note also the hypertrophy and hyperplasia in the vascular parenchyma (VP).

Figure 27.—Field study. Hydrogen fluoride, Scotch pine, longitudinal section, X125. These specimens were obtained from the Rhone Valley in Switzerland. Characteristic of injury caused by fluoride and other phytotoxic gases, endodermal cells (EN) in contact with necrotic mesophyll (MP) are necrotic, the vascular cylinder (VC) is deeply stained, and vascular parenchyma (VP) has hypertrophied. Compare with figures 14 through 19 and 23 through 26.

Figure 28.—Field study, hydrogen fluoride, Scotch pine, transverse section, X125. This specimen also is from the Rhone Valley in Switzerland. The section was cut in the greenish portion of the transition zone. Notice the deep staining of vascular parenchyma (VP).
and laboratory-induced stress were similar, indicating that the symptoms caused by various agents as well as by plants under controlled conditions are: 1. Collapse of endodermal cells with collapsed mesophyll. 2. Hypertrophy and hyperplasia of vascular parenchyma. 3. Deep staining of vascular tissues, extending basipetally into the region of healthy mesophyll.

Winter damage, drought, and salt induced the following symptoms:

1. Mesophyll cells collapse; but endodermal cells are not affected, even when in contact with necrotic mesophyll.
2. Vascular parenchyma collapse; hypertrophy and hyperplasia are not evident.
3. Deep staining of vascular tissues does not occur. Symptoms in field-collected specimens representative of a variety of conifers were similar for similar causal agents.

Identification of the cause of conifer injury and related forest damage near sources of phytotoxic air emissions may be confounded by insects, disease, weather, or other abiotic agents. Affected trees usually exhibit foliar chlorosis and necrosis in response to causal factors. This study indicates that through histological procedures these causal factors may be distinguished. When used in conjunction with ambient air quality data, emission data, foliar chemical analyses, and observations of the status of biotic plant pathogens and foliage-feeding insects, histological observations should strengthen the diagnosis of cause in damage surveys done near polluting industries.

PUBLICATIONS CITED


Symptoms induced by phytotoxic gases within conifer needles can be differentiated histologically from those caused by other abiotic agents including winter drying, drought, and salt. However, it is not possible to differentiate among symptoms caused by hydrogen fluoride, sulfur dioxide, ethyl mercaptan, and hydrogen sulfide. Phytotoxic gases cause hypertrophy and hyperplasia of vascular parenchyma, endodermis collapse, and intense vascular staining. The other abiotic agents induce mesophyll collapse with little or no observable effects on vascular tissues. Histological analyses should be useful in diagnosis of air pollution-induced injury and damage in coniferous forests.

KEYWORDS: phytotoxic gases, winter injury, conifer needles, symptoms, histology, air pollution, diagnosis, needle necrosis